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≪Research Note≫

Glucose Tolerance and Plasma Non-Esterified Fatty Acid Levels in Chickens Selected for Low Body Weight, Red Junglefowl, and their Reciprocal Cross

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Responses of an individual to food deprivation, such as a 16-h fast, are complex, and are influenced by environmental and genetic factors. Domestication is an ongoing process during which adaptations to changing environments occur over generations. Food deprivation by their caretakers is less for domestic chickens than for their junglefowl ancestors. Unlike domestic chicken, the junglefowl adapted over generations to periods of food deprivation, which may be reflected in differences in metabolic responses to brief periods without food. Here, we compared the blood glucose and plasma levels of non-esterified fatty acids (NEFA) among four populations when deprived of feed for 16 h. The four populations included a domestic White Rock experimental line (LWS) maintained for generations under *ad libitum* feeding, adult red junglefowl (RJF), and a reciprocal cross of the lines. Although there were significant differences in adult (31-week) body weight between the RJF (683 g) and LWS (1282 g), with the weight of F_1 crosses being intermediate, the amount of abdominal fat relative to body weight was similar for all populations. Patterns for blood glucose responses to a glucose bolus after a 16-h fast were similar for the initial and final points in the parental and cross populations. However, RJF reached their peak faster than LWS, with the reciprocal cross intermediate to the parental populations. Plasma NEFA concentrations were higher after the 16-h fast than in fed states, with no population differences for the fasting state. However, in the fed state, NEFA levels were lesser for LWS than for others, which was reflected further in percentage change from fed to fasted. This larger change in LWS suggests differences in mobilization of energy substrates and implies that during domestication or development of the LWS line, thresholds for responses to acute stressors may have increased.

Key words: blood glucose, chickens, heterosis, plasma NEFA, reciprocal cross

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Introduction

Certain behaviors that enhanced the reproductive success and survival of the junglefowl in its natural habitat have, as a result of human-driven selection and husbandry, become redundant in the domestic chicken. Examples of behavior that are still probably present in domestic chicken include escape, incubation, and maternal behaviors, although the thresholds for the responses may have been increased. During the course of an experiment involving red junglefowl (RJF) and a line of White Plymouth Rocks selected long-term for low juvenile (56 day) body weight (LWS), we observed that under the same husbandry, the former was exceptionally flighty, whereas the later were docile in their response to humans (Sutherland *et al*., 2018). This observation provides an example of behavioral redundancy, namely fear from predation. Acute stress responses and prompt mobilization of energy, per Cannon's fight or flight response, are involved in escape behaviors, which have been elaborated numerous times in higher vertebrates (Goligorsky, 2001) including chicken (Siegel, 1980; Broom and Johnson, 1993; Appleby *et al*., 2004). For example, a burst of energy requires elevated blood glucose levels to respond to short-term stressful situations.

In stress response, the adrenal cortex is stimulated to release glucocorticoids, which stimulate glucose synthesis and the redistribution of stored fat for long-term energy require-

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ments (Heald *et al*., 1965; Lepkovsky *et al*., 1967, Hazelwood, 1971). The abdominal adipose depot is a major source of non-esterified fatty acids (NEFA), which can provide energy as fatty acids or glycerol during stress such as food deprivation or high activity (McWilliams *et al*., 2004; Nielson *et al*., 2004). Contrary to mammals, chickens are relatively insulin-resistant and exhibit a low glycemic-high adiposity balance. Despite the presence of normal circulating insulin, plasma IGF-I and insulin levels were higher, whereas plasma glucose level was lower in chickens from a line selected for high body weight, the abdominal fat percentage of which was 12 times higher than those of chickens from its companion line selected for low body weight (Beccavin *et al*., 2001; Nadaf *et al*., 2007). This pattern was more pronounced in lines divergently selected for fasting glycemia (Leclercq *et al*., 1987).

Oral glucose tolerance tests are used to assess the pancreatic content of insulin and can be used to characterize differences in hyperglycemic response. While investigating glucose regulation, Sumners *et al*. (2014) observed differences in threshold sensitivity to insulin and glucose clearance rates in lines of chickens divergently selected for high and low juvenile (56 day) body weight. Comparisons of glucose tolerance and plasma NEFA concentrations among RJF, LWS selected for > 50 generations for low body weight, and their reciprocal cross may provide insights regarding metabolic responses. This will also provide information on energy substrate mobilization and possible hereditary influences. Sutherland *et al*. (2018) reported intra- and intergenerational differences in growth, reproduction, and behavioral traits between the domesticated LWS, their RJF progenitor, and reciprocal F_1 cross. These populations provide a model to study the genetics of complex traits in general and the effects of artificial selection on metabolic processes following acute food deprivation. Here, we compared glucose homeostasis and metabolic responses among LWS, RJF, and their reciprocal F_1 cross.

Materials and Methods

All procedures were performed in accordance with the guidelines approved by the Virginia Tech Institutional Animal Care and Use Committee.

Experimental Populations

The foundation populations used in this study were pedigreed White Plymouth Rock chickens selected for low 56-day body weight (LWS, generation 57; Dunnington *et al*., 2013; Jambui *et al*., 2017a) and the Richardson strain of red junglefowl (RJF; Brisbin and Peterson, 2007). To form the LWS line, individuals with lower body weights at 56 days of age were selected from a segregating gene pool that consisted of crosses of seven moderately (12−36%) inbred lines of White Plymouth Rocks (Bywaters and Siegel, 1958; Siegel, 1962). Thereafter, with some restrictions (e.g. size of sire and dam families and avoidance of sibling matings), the single criterion of selection used was low 56-day body weight. The numbers of sires and dams selected to reproduce the LWS line were eight and 48, respectively, through generation 4 (S4). From S5 to S25, the numbers of sires and dams were 12 and 48, respectively. For the S26 and subsequent generations, matings involved 14 sires and 56 dams. Details concerning population structures, inbreeding, and scaling are described by Marquez *et al*. (2010) and Jambui *et al*. (2017a). The Richardson strain of RJF included descendants from RJF originally collected in India in the vicinity of Dehradun during the 1960s (Brisbin and Peterson, 2007) and has been maintained in genetic isolation as 10 to 50 individuals. In 2013, two males and four females were transferred from the Brisbin flock to the same facility as the LWS where we generated a flock of 10 males and 17 females.

A pedigreed reciprocal cross (F_1) was generated by crossing LWS males with RJF females to produce the LR progeny, and RJF males were mated with LWS females to produce the RL progeny. The parental populations were selected for the study as they differed considerably in physical and behavioral traits (Sutherland *et al*., 2018). For example, the RJF is more fearful of humans and has smaller adult body mass and secondary sex characteristics (comb weight, height, and length) than LWS. Although breast weights are similar for RJF and LWS, they are larger for RJF than for LWS when expressed as a percentage of body weight.

Husbandry

Across generations, artificial insemination, incubation, and hatch dates were scheduled each year such that chicks hatched on the first Tuesday in March. Upon hatching, the chicks were wing-banded for individual pedigree identification, vaccinated for Marek's disease, and placed in pens within battery brooders with constant light. To reduce competition, the populations were not allowed to intermingle. Subsequently, sexes within a population were separated for the same reason. At 8 weeks of age, the chickens were transferred to pens with concrete floors covered with pine wood shavings for litter and a natural day length photoperiod. At 20 weeks of age, they were placed in individual cages in a room with a 10:14 photoperiod of dark: light. The dietary formulation fed throughout this two-generation experiment consisted of 20% crude protein and 2,685 kcal ME/kg from hatch to 8 weeks of age. From 9 to 20 weeks, the diet consisted of 16% crude protein and 2,761 kcal ME/kg, and thereafter 16% crude protein and 2,772 kcal ME/kg. Throughout all generations and ages, feed in mash form and water were provided *ad libitum*.

Measurement of Traits and Statistical Analysis

Adult body weights (g) were obtained at 31 weeks of age, at which time all chickens were euthanized and abdominal fat was removed and weighed (g). Abdominal fat consisted of fat surrounding the gizzard, adjacent abdominal muscles, and the ventral side of the ischium.

For the oral glucose tolerance test (OGTT), blood glucose data were obtained for 61 adult females that were randomly assigned within a genetic population to one of two treatment groups: glucose (LR-4; LWS-10, RJF-10; RL-7) or vehicle (LR-4; LWS-9, RJF-10; RL-7). After a 16-h fast, each individual was weighed (g) and administered a glucose bolus (2 g/kg body weight; 20% weight/volume H_2O) or an equiva-

lent volume of water via oral gavage. Blood glucose concentrations of chickens receiving the glucose bolus were measured at 0, 5, 15, 30, 60, 120, and 240 min and at 0, 5, 15, and 30 min for vehicle-treated chickens. A small drop of blood was obtained by pricking a small brachial blood vessel with a 23-gauge needle, collected, and read using a handheld glucometer (Agamatrix, Inc., Salem, NH, USA), following the method described by Zhao *et al.* (2012).

Data were analyzed using the Fit model or Fit Y by X platforms (JMP, 2015). Analysis of variance (ANOVA) was used to analyze the main effects such as population, time, and the interaction between them using the formula $\boldsymbol{Y}_{ijk}=\mu+\boldsymbol{P}_i+\boldsymbol{T}_j+\boldsymbol{PT}_{ij}+e_{ijk}$, where $i=1, 2, 3, 4$ genetic populations, $j=0, 5, 15, 30, 60, 120$, and 240 min for those administered glucose, and $k=1, 2 \ldots n$ individuals; for those administered water, $j=0, 5, 15$ min. When the population by time interaction was significant, comparisons were made between time points within a population and between populations for a specific time point. Retrospectively, due to lack of differences in the previous ANOVA for baseline readings, we pooled baseline readings for the control and glucose groups within each population (LR-8; LWS-19, RJF-20; RL-14) and conducted a one-way ANOVA for the baseline (time 0) using the formula $\boldsymbol{Y}_{ij}=\mu+\boldsymbol{P}_i+e_{ij}$, where $i=1, 2, 3, 4$ genetic populations and $j=1, 2\ldots n$ individuals. Similarly, this model was used to compare populations for fasted body weight.

Calculations for area under the response curve followed the procedure described by Gilbert *et al.* (2011) according to the trapezoid rule. Incremental areas under the curve were calculated for each individual as the sum of blood glucose measurements for two consecutive time points, multiplied by the time interval, and then divided by two. In addition, glucose clearance rates were calculated for each individual from OGTT data by determining the slope of the line $(m=y^1-y^2).(x^1-x^2)^{-1})$ for blood glucose measurements between individual peak points and 120 min. This procedure was necessary because of differences among populations with respect to time to peak. The ANOVA model for area under the curve, glucose clearance rates, and time at peak was represented by the formula $\boldsymbol{Y}_{ij}=\mu+\boldsymbol{P}_i+e_{ij}$, where $i=1, 2, 3, 4$ genetic populations and $j=1, 2,\ldots n$ individuals.

Plasma NEFA levels were measured at 28 weeks of age in 61 adult females (LR-8; LWS-19, RJF-20; RL-14). Each female was tested in the fed and fasted states. Prior to and immediately following a 16-h fast, approximately 200 μL blood was obtained from the brachial vein in capillary blood collection tubes (Microvette®). Samples were then centrifuged at 2,000 × *g* at room temperature for 2 min and plasma was collected. Plasma NEFA concentrations were measured using the NEFA-HR2 kit (Wako Diagnostics, Mountain View, CA) according to the manufacturer's instructions. Absorbance was measured at 550 nm using an Infinite M200 Pro multi-mode plate reader (Tecan). Sample concentrations were calculated as standard concentration × sample absorbance standard absorbance.

As the experiment involved only females, the ANOVA main effects were population, fasted/fed state, and the interaction between them, which is expressed by the formula $\boldsymbol{Y}_{ijk}=\mu+\boldsymbol{P}_i+\boldsymbol{F}_j+\boldsymbol{PT}_{ij}+e_{ijk}$, where $i=1, 2, 3, 4$ genetic populations, $j=$fed, fasted state, and $k=1, 2 \ldots n$ individuals. As the interaction of population and fed/fasted state approached significance ($P=0.06$), we made a *post facto* assumption that there was an interaction. Therefore, a one-way ANOVA was conducted to compare populations within the fasted and fed states. The same ANOVA model was used to analyze body weight, abdominal fat, plasma NEFA levels, percentage differences in plasma NEFA levels, and abdominal fat as a percentage of body weight. Product moment correlations were conducted within each population for plasma NEFA levels with blood glucose, body weight, and abdominal fat, as well as for abdominal fat with body weight.

Percentage differences in plasma NEFA = [(Fasted NEFA − Fed NEFA) / Fed NEFA] × 100

Abdominal fat as a percentage of body weight = (Abdominal fat / body weight) × 100

Heterosis = {[LR or RL − (LWS + RJF) / 2] / [(LWS + RJF) / 2]} × 100

Prior to analyses, body weights were log transformed as means and variances were correlated, and percentages were transformed to arcsine square root. Significance was considered at $P<0.05$.

Results

Comparison among Populations

There were significant differences among populations for body weight, with LWS being heaviest, RJF being lightest, and crosses being similar to each other, intermediate to the parental lines (Table 1). Although abdominal fat weights differed significantly between the parental lines, with that of LWS being more than twice that of RJF, they did not differ when expressed as percentage of body weight. The abdominal fat content of LR, both absolute and relative to body weight, was significantly higher than that of RL. Absolute abdominal fat content followed a pattern, where the content in LR was similar to that in LWS, whereas the content in RL was similar to that in RJF. The percentage body weight of RL was lesser than that of RJF, whereas the percentage of abdominal fat of LR was more than those of the other populations. Heterosis for abdominal fat was large, with opposing signs for the reciprocal crosses, as those of RL and LR were −60% and +62%, respectively (Fig. 1). Correlations between abdominal fat and body weight for RJF, LWS, RL, and LR (r=0.27; r=0.54; r=0.34; r=0.65, respectively), were positive, but not significant.

Plasma NEFA levels were significantly higher in the fasted than in the fed state in all populations (Table 1). In the fed state, values for LWS were significantly lower than those for all other populations, which did not differ among themselves. In contrast, there were no differences among populations in the fasted state. However, comparisons of fasted versus fed states within populations revealed significant differences between satiety levels in all populations. Further analysis of the percentage change between plasma NEFA levels in fasted and fed states revealed that the difference was

Table 1. **Means and standard errors by population for adult female chicken body weight, abdominal fat (absolute and relative to body weight), and plasma non-esterified fatty acids (NEFA) (in fasted and fed states and percentage difference between states)**

Population[1]	n	Body weight (g)	Abdominal fat		Plasma NEFA		
			Absolute (g)	Relative[3] (%)	Fasted state (mEq/L)	Fed state (mEq/L)	Difference[2] (%)
RJF	20	683±20[c]	8.9±1.4[b]	1.3±0.2[b]	0.53±0.03[a]**	0.33±0.02[a]	38±4[b]
RL	14	902±21[b]	6.1±1.4[b]	0.7±0.2[c]	0.47±0.03[a]**	0.35±0.03[a]	27±5[b]
LR	8	992±49[b]	24.8±4.0[a]	2.4±0.4[a]	0.55±0.04[a]*	0.43±0.03[a]	22±6[b]
LWS	19	1282±31[a]	21.6±2.8[a]	1.7±0.2[b]	0.45±0.03[a]**	0.19±0.02[b]	55±4[a]

[a–c] Means in a column with no common superscript differ at $P<0.05$. For plasma NEFA levels, comparisons between fasted and fed states (* $P<0.05$ and ** $P<0.01$).

[1] RJF represents the red junglefowl population and LWS (generation 57) represents the line of chickens selected for low body weight. For each F_1, the first letter designates the sire line and the second designates the dam line.

[2] Difference in plasma NEFA levels in fasted and fed state=[(fasted NEFA−fed NEFA)/ fasted NEFA]×100.

[3] Abdominal fat expressed as a percentage of body weight=(abdominal fat / body weight)×100.

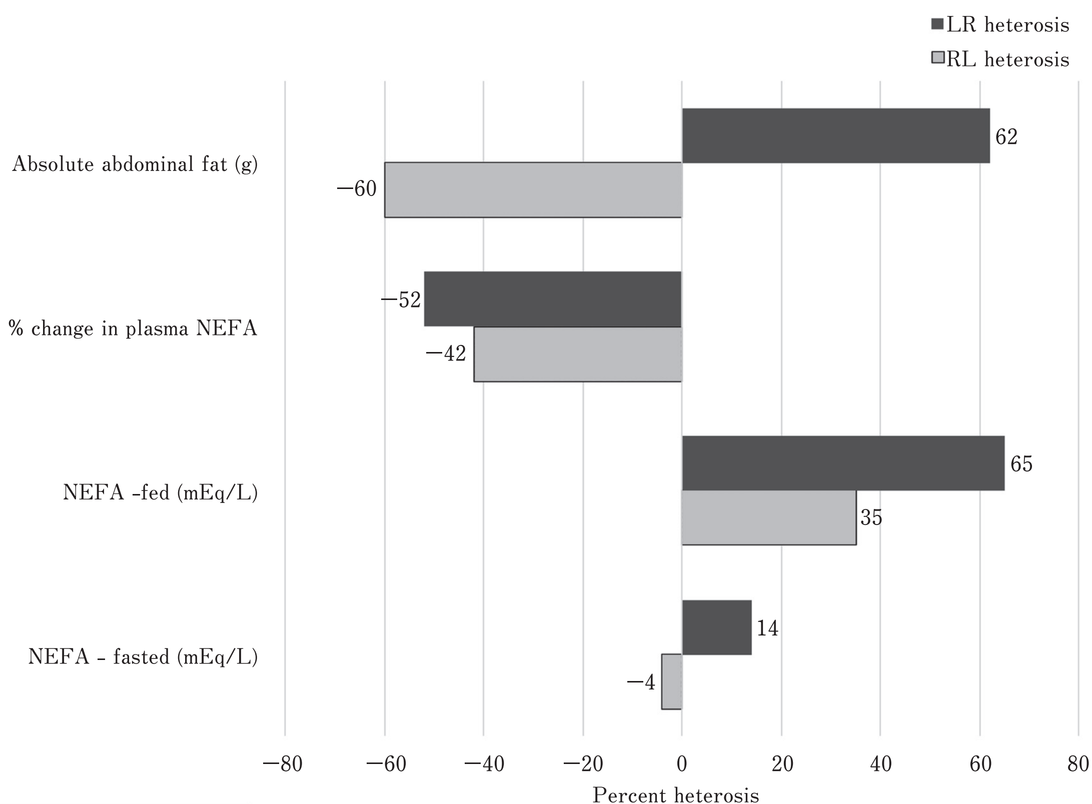
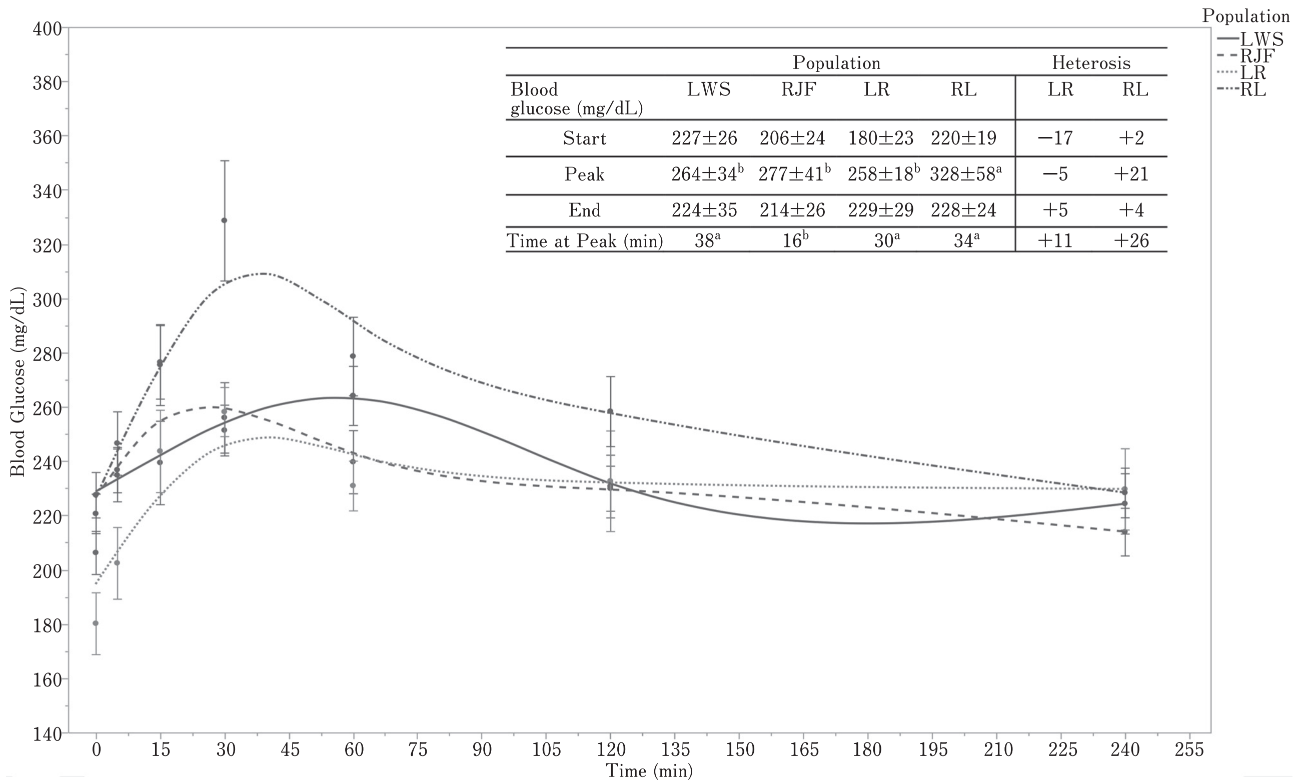


Fig. 1. **Percentage of heterosis for absolute abdominal fat weight, percentage change in plasma non-esterified fatty acid (NEFA) levels, and NEFA levels in fed and fasted states for adult female chickens in a reciprocal F_1 cross.**

significantly larger for LWS than for the other populations, which were similar. Heterosis for the percentage change in plasma NEFA levels between the two states was similar, being −52% and −42% for LR and RL, respectively. Heterosis for plasma NEFA level in the fed state was large and positive, being 65% and 35% for LR and RL, respectively. In contrast, in the fasted state, heterosis was 14% and −4% for LR and RL, respectively (Fig. 1). Plasma NEFA level and body weight did not correlate significantly in RJF, LWS, RL, and LR (r=0.08; r=0.24; r=−0.18; r=−0.12, respectively). Similarly, plasma NEFA and abdominal fat did not correlate for RJF, LWS, RL, and LR (r=0.16; r=−0.05; r



	Population				Heterosis	
Blood glucose (mg/dL)	LWS	RJF	LR	RL	LR	RL
Start	227±26	206±24	180±23	220±19	−17	+2
Peak	264±34[b]	277±41[b]	258±18[b]	328±58[a]	−5	+21
End	224±35	214±26	229±29	228±24	+5	+4
Time at Peak (min)	38[a]	16[b]	30[a]	34[a]	+11	+26

Fig. 2. **Blood glucose trends for parental and reciprocal F_1 cross populations, and percentage of heterosis. LWS (generation 57) represents the chicken line selected for low body weight; RJF represents red junglefowl.** For the reciprocal cross, the first letter designates the sire line, and the second designates the dam line. Means with different superscripts within a row differ at $P<0.05$.

=−0.19; r=0.03, respectively).

Within the vehicle group, there was no population, time, or population by time interactions in the OGTT. At time zero, the OGTT result was 208±10 mg/dL. Accordingly, all subsequent analyses were conducted for chickens receiving the glucose bolus. The patterns differed among populations (Fig. 2). The initial blood glucose level of LWS chickens was 227±26 mg/dL, which peaked, on average, at 38 min (264±34 mg/dL) and ended at 224±35 mg/dL; in contrast, the initial blood glucose level of RJF chickens was 206±24 mg/dL, which peaked at 16 min (277±41 mg/dL) and ended at 214±26 mg/dL blood glucose. The blood glucose curve was highest for RL, which started at 220±19 (mg/dL), peaked at 34 min (328±58 mg/dL), and ended at 228±24 mg/dL. In contrast, LR had the lowest initial blood glucose level (180±23 mg/dL), lowest peak at 30 min (258±18 mg/dL), and final point at 229±29 mg/dL. Heterosis for oral glucose tolerance (Fig. 2) was divergent for both the starting point (−17% and +2%) and peak (−5% and +21 %) for LR and RL, respectively. Heterosis for the end point was +5% and +4%, and time to peak was +11 and +26 for LR and RL, respectively. Baseline blood glucose values and plasma NEFA levels did not correlate for RJF, LWS, RL, and LR (r=0.15; r=−0.40; r=−0.18; r=0.16, respectively).

The area under the curve, which was calculated for the 15 to 120 min period for glucose-treated birds, was similar for all populations (Table 2). However, there were differences among populations in the control group. The area under the curve was largest for RJF and smallest for LWS, with the reciprocal cross being similar to each other and intermediate to their parental populations. This uniformity was reflected in the low heterosis, being −2% and −3% for controls and 11% and −1% for glucose-administered RL and LR chickens, respectively. Glucose clearance rates did not differ among populations for RJF, LWS, RL, and LR (0.57±0.09, 0.78±0.10, 1.09±0.31 and 0.44±0.15, respectively).

Discussion

Via domestication, chicken evolved from a natural habitat to that where humans assumed various roles relative to their survival. Over time changes have behavioral and physiological processes as well as anatomical features, some of which have been addressed in our experiment. For example, although absolute abdominal fat weight of RJF was less than half of that of LWS, they were similar (<2%) when ex-

Table 2. **Means, standard errors, and percentage heterosis in adult female chickens for glucose clearance rates, and area under the curve (AUC) for control and glucose-treated groups by population**

Treatment group		Population[1]				Heterosis[3]	
		RJF	LWS	RL	LR	RL	LR
Control	AUC	11887±454[a]	9607±454[b]	10566±542[ab]	10426±717[ab]	−2	−3
Glucose [2]	AUC	55810±1942[a]	57212±1843[a]	62675±2202[a]	55954±2913[a]	+11	−1
Glucose [2]	Glucose clearance rate[4]	0.57±0.09[a]	0.78±0.10[a]	1.09±0.31[a]	0.44±0.15[a]	+70	−31

[a–b], Means in a row with no common superscript differ at $P<0.05$.
[1] RJF represents the red junglefowl population and LWS (generation 57) represents the line of chickens selected for low body weight. For each F_1, the first letter designates the sire line and the second designates the dam line.
[2] Chickens received a glucose bolus (2 g/(kg body weight); 20% weight/volume H_2O).
[3] Percentage heterosis was calculated as {[LR or RL−(LWS+RJF) / 2] / [(LWS+RJF) / 2]}×100.
[4] Glucose clearance rate (mg/dL/min) was calculated by determining the slope of the line ($m=(y^1-y^2)\ .\ (x^1-x^2)$) for blood glucose measurement between time of peak for each population to 120 min.

pressed relative to body weight. There were indications of a paternal effect for absolute abdominal fat weight in females, as the F_1 birds (LR and RL) were phenotypically similar to their paternal line rather than to each other. This pattern, however, was not evident when expressed relative to body weight, as there was no difference between the parental lines. The value for LR F_1 females exceeded that of the parental lines, which then exceeded the value for RL. This lack of balance was reflected in the heterosis for absolute abdominal fat content, which was strongly divergent (+62% for LR and −60% for RL). This pattern suggests that one or more Z-linked genes may be involved in the inheritance of abdominal fat, because the hemizygous LR females receive their Z chromosome from their LWS sire, whereas all RL females harbor a Z chromosome derived from their RJF sire. Sutherland *et al.* (2018) reported similar results while studying intra- and intergenerational differences in growth, reproduction, and behavioral traits between the domesticated LWS, their wild progenitor RJF, and reciprocal F_1 and F_2 crosses. Furthermore, absence of differences in abdominal fat weight between the female F_2 reciprocal crosses provided additional support to this possibility, as the F_2 had equal likelihoods of inheriting alleles originating from either LWS or RJF at a certain Z-linked locus.

Although chickens have numerous fat depots (subcutaneous, clavicular, and hepatic), we focused on the abdominal fat depot as it is a well-studied and major source of NEFA. Abdominal fat as a percentage of body weight averaged 1.5% across the parental and F_1 populations, which was consistent with the results of previous studies on a range of populations. For example, the ratio of average abdominal fat to body weight was 1.53% and 1.49% in Ross x Cobb broilers (McNaughton *et al*., 2007) and 1.47% for female Ross x Ross broilers (McNaughton *et al*., 2008). For commercial broiler hens, relative abdominal weights of 2.2% and 2.1% were calculated from data reported by Chen *et al*. (2006) and Jiang *et al.* (2016), respectively. In addition, Renden and Marple (1986) reported values of 3.1% and 2.1% for Dwarf White Leghorns divergently selected for high and low body weight. Although absolute abdominal fat weights for chickens in these studies varied, the relative weight of abdominal fat to body weight was similar and was consistent with our data. The consistency of abdominal fat to body weight ratio across different breeds and populations over many generations leads to speculations regarding the existence of homeostatic mechanisms for balancing fat deposition and skeletal muscle formation. It also suggests that this mechanism has been conserved during the domestication process, as RJF had the same ratio as the domesticated populations.

However, the balance between muscle and fat tissue can be disrupted in cases of artificial selection where the focus is on yield or growth *per se* with little or no regard for adipose tissue. In the 17th generation of the Slovenian Prelux meat type chickens divergently selected for body weight at 8 weeks of age, Holcman *et al*. (1995) reported a higher share of abdominal fat from live weight in their high weight line than in the low weight line. In our long-term selection experiment for high and low body weight at 8 weeks of age (Siegel, 1962; Marquez *et al*., 2010; Jambui *et al*., 2017b) lines differed by >15 fold in the selected trait. Most of these differences were due to changes at many loci, with each having small additive effects (Jacobsson *et al*., 2005). Lillie *et al*. (2018) reviewed the genomic signatures of these lines, which differ in percentage of adipose tissue as well as in abdominal fat (Sutherland *et al*., 2018). The increased adiposity, heavier fat pads, and higher percentage carcass fat was also observed in comparisons of 1957 and 1991 broilers that were of the same age and were fed the same diet (Havenstein *et al*., 1994). This imbalance was correctable, as abdominal fat has high heritability, which allowed breeders to select for lower abdominal fat via sib-matings. Within a few generations, the breeders achieved a change with ratios of <2% (Gaya *et al*., 2005) which restored the natural balance.

Adipose depots provide energy as glycerol and fatty acids in times of stress, nutrient deprivation, or high activity. Proteins involved in lipolytic pathways and processes are highly regulated at multiple levels across species (Bernlohr *et al*., 2002). The ability of adipose tissue to switch efficiently between catabolic and anabolic states is important for

survival. The difference between RJF and LWS with respect to plasma NEFA levels in the fed state and percentage difference in plasma NEFA levels indicate differences in the modulation of energy substrate mobilization. Logically, availability of free fatty acids for use in a burst of energy during acute stress, such as to escape predation, might be beneficial for an organism. For instance, Sutherland *et al*. (2018) reported that RJF were flighty and difficult to handle, and thus more fearful of humans than LWS. Heterosis for plasma NEFA levels in the fed state was +65% for LR and +35% for RL, whereas heterosis for plasma NEFA levels in the fasted state was +14% for LR and −4% for RL. However, heterosis for the change in plasma NEFA level between the two satiety levels was rather similar, being −52% for LR and −42% for RL. These observations provide evidence for the existence of both additive and non-additive genetic effects for conservation of lipolytic pathways and processes associated with NEFA availability. Concomitant with our results, Langslow *et al*. (1970) reported increased plasma NEFA levels in chickens and decreased glucose levels after fasting (72 h). Correspondingly, plasma NEFA levels decreased after eating, whereas glucose levels increased.

Although glucose response patterns were similar for parental populations and their reciprocal crosses with respect to the initial and final points, glucose level of LWS peaked later than that of RJF, with the reciprocal cross intermediate to the parental lines. The rate of change in blood glucose from start to peak was slowest for LWS, fastest for RJF, and intermediate for the reciprocal F_1 cross. The RJF mounted sharp and rapid response during the first 16 min, whereas LWS exhibited a more gradual increase to peak at 38 min. Glucose clearance rates for a fixed physiological period from peak to 120 min followed a different pattern among the populations. The variation in the time to peak rather than in the time to mitigate a surge of blood glucose through metabolic processes suggests differences among the populations, both in neural aspects and metabolic processes such as nutrient uptake via intestinal absorption. Hence, LWS chickens may have altered thresholds for glucose oxidation via insulin release, which is evident from the shape, duration, time to peak, and time for the re-attainment of normal blood glucose levels (Sturkie, 1965). Analogous to this pattern of response, Duncan (1979) reported that the heart rate of a “flighty” strain of chickens returned to normal after visual and auditory frightening sooner than that of a more “docile” strain.

Genetic factors affect the allocation of an animal’s resources to various components such as growth, reproduction and health status (Siegel and Gross, 2007; Jambui *et al*., 2017b). Domestication can disrupt the response threshold, which is usually low for wild animals. In contrast to variations within and across populations, the genome of an individual remains constant (barring somatic mutations). Lifetime experiences, sex, age, reproductive status, and the genome environment can influence the phenotypic responses of an individual to acute stressors or outcomes to experiences in general. Comparisons involving metabolic responses such as glucose homeostasis are complicated. For example, Panigrahy *et al*. (2017) reported sex (males > females) and seasonal (winter > summer) effects on blood glucose levels. Sumners *et al*. (2014) reported blood glucose values for juvenile LWS females to be higher than those obtained here for females in lay. These results, in concert with the lack of correlation between body weight, abdominal fat, blood glucose, and plasma NEFA levels, suggest a balanced response to the brief fast of 16 h. The degree of response and ability to restore equilibrium suggest that during the history of the species (domestication in this case), population, individual thresholds to responses, and the responses *per se* may vary. Thus, although genotype-environment relationships are complex, equilibrium patterns emerge when viewed as aims and strategies for survival. Although the domestic chicken is far removed from its red junglefowl ancestor, their strategies for maintaining metabolic homeostasis remain similar.

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